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IN VITRO DEVELOPMENT OF INSECT TISSUES. II. THE ROLE OF ECDYSONE IN THE SPERMATOGENESIS OF SILKWORMS [1]

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In the preceding paper of this series (Kambysellis and Williams, 1971) we confirmed the earlier observations of Schmidt and Williams (1953) that a biologically active "macromolecular factor" (MF) is present in the hemolymph of male and female silkworms. MF is recognizable in terms of its ability to provoke the meiosis and spermatogenesis of germinal cysts removed from diapausing testes and cultured *in vitro*.

By the use of this biological assay the blood of Cynthia pupae was found to contain substantial MF activity throughout all but a few weeks of pupal diapause. Yet, strange to say, the germinal cysts within the testes of diapausing pupae undergo no developmental response until such time as diapause is terminated in response to ecdysone injection or the secretion of ecdysone by the insect's own prothoracic glands.

This paradox is examined in detail in the studies reported here. Our experimental approach was to examine the effects of MF and ecdysone on *in vitro* cultures of intact testes. The results were strikingly different from those previously reported for the naked germinal cysts.

Materials and Methods

All experiments were carried out on diapausing pupae of the Cynthia silkworm (*Samia cynthia*). The experimental procedures were precisely the same as described by Kambysellis and Williams (1971) except that the *in vitro* technique was modified for the culture of intact testes.

Each culture chamber consisted of a depression slide (spherical concavity 18 mm in diameter and 1.5 mm in depth), a Teflon spacer ring 2.5 mm in thickness (cut from 25.3 mm O.D., 19.0 mm I.D. tubing), and a No. 1 circular glass coverslip 22.0 mm in diameter. All components were sterilized overnight at 140° C.

To prepare the culture, 100 μl of an appropriate medium was pipetted into the concavity of the slide and an intact testis, cleaned-up and rinsed as previously described (Kambysellis and Williams, 1971), was added. The testis was handled by grasping an attached fragment of trachea with sterile forceps. The Teflon spacer ring was placed in position and capped with the cover slip. The assembled chamber was then sealed by brushing melted wax around its edges.

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The samples of α-ecdysone and phytoecdysones were obtained through the courtesy of Dr. John Siddall of Zoecon Corporation, Prof. K. Nakanishi of Columbia University, and Prof. T. Takemoto of Tohoku University. Mention is also made of an experiment using tritiated ecdysone; this material was synthetic α-ecdysone-23,24-^{3}H (40 c/mmole) which was most kindly supplied by Dr. Siddall.

Results

1. *Development of germinal cysts* in vitro *and* in vivo

(a) *Effects of MF.* In a series of control experiments the titer of MF was assayed in hanging-drop cultures prepared from the blood plasma and naked germinal cysts of each of ten normal, diapausing, male Cynthia 2 to 3 months after pupation. As anticipated for pupae of this age-class (Kambysellis and Williams, 1971), the assays confirmed the presence of a low titer of MF as signaled by the development of only 5 ± 4% of the germinal cysts (Table I). The experiment was repeated on ten additional pupae which had been stored at 25° C for 5 to 6 months. As anticipated for pupae of this age, substantial MF activity was revealed by the assays (25 ± 5%).

Microscopic examination of the freshly prepared cultures revealed no trace of development of the cysts immediately after their removal from the testes. In these and scores of similar preparations from diapausing pupae, meiosis and spermatogenesis of naked cysts began only when they were cultured in direct contact with MF.

(b) *Effects of integumentary injury.* The experiment was repeated on twelve pupae that had been stored at 25° C for 2 to 3 months. In this case, each individual was transected with a razor blade just behind the metathorax to provide isolated

Table I

Effects of injury and of ecdysone injection on the development of the germinal cysts in vivo *and* in vitro

Treatment	Number of pupae	Hours after treatment	% of cysts developing		
			In the intact testes *in situ*	In cultures of the pupa's own cysts and plasma	In plasma cultures of cysts from untreated pupae
None (controls)	10	—	0	5 ± 4(53)†	—
	10*	—	0	25 ± 5(45)	—
Injured (abdomens isolated)	6	72	0	56 ± 5(18)	65 ± 9
	6	96	0	41 ± 7(18)	43 ± 6
Inject 10 μg α-ecdysone	5	1	0	—	3 ± 2
	5	24	[Stage II only]	—	25 ± 6
	5	48	10–20	83 ± 6(17)	33 ± 8
	5	72	40–50	86 ± 7(20)	45 ± 11

* These 10 pupae were 5–6 months after pupation; all the others were 2–3 months.
† Number of cultures are recorded in parentheses.

abdomens which had sustained massive integumentary injuries. Crystals of an equal part mixture of phenylthiourea and streptomycin sulfate were placed in the wound along with sufficient blood from the anterior fragment to displace all air. Each isolated abdomen was then sealed with melted wax to a plastic slip.

After 72 hours six of the preparations were sacrificed. The hemolymph and testes of each individual were collected and plasma cultures of the germinal cysts were prepared in the usual manner. In parallel assays an aliquot of each sample of plasma was tested for MF titer on germinal cysts obtained from normal, uninjured, diapausing pupae. These same manipulations were carried out on the second group of six abdomens which were sacrificed 96 hours after their isolation.

As indicated in Table I, all samples of plasma from the injured, isolated abdomens showed high MF activity irrespective of whether they were assayed on their own germinal cysts or on cysts obtained from uninjured diapausing pupae.

(c) *Effects of ecdysone.* Table I summarizes a further series of experiments carried out on twenty Cynthia 2 to 3 months after pupation. At zero hours each individual was injected with 10 μg of α-ecdysone. Groups of five individuals were sacrificed at specific periods after injection and treated as described above for the isolated abdomens.

The samples of plasma, when assayed on the germinal cysts of normal uninjected pupae, showed a substantial increase in MF titer within 24 hours and further increases during the succeeding two days. The cysts harvested from the testes of the injected pupae showed no development 1 hour after injection. But after 24 hours the testes contained many cysts which had completed meiosis (Stage II). By 48 to 72 hours after injection the testes contained steadily increasing numbers of cysts showing advanced spermatogenesis (Stages III and IV). As indicated in Table I, nearly all of these germinal cysts completed spermatogenesis when cultured for 7 days in the MF-containing plasma of the injected individuals.

These several experiments confirm a conclusion already documented by Kambysellis and Williams (1971)—namely, that naked cysts require MF for their development; as previously shown, the presence or absence of ecdysone is inconsequential in cultures of naked cysts. The present experiments go on to show that cysts within the intact testes of normal or injured pupae cannot respond to MF unless ecdysone is also present. These findings raised the possibility that ecdysone promotes the entry of MF into the cavity of the testes. This hypothesis was subjected to direct examination in the experiments which follow.

2. *In vitro culture of intact testes: effects of MF and ecdysone*

Individual testes of normal, diapausing Cynthia were cultured: (1) in medium containing MF but no ecdysone; (2) in medium containing ecdysone but no MF; and (3) in medium containing both MF and ecdysone. After 7 days the testes were torn open and examined microscopically for the development of the germinal cysts. As summarized in Table II, the cysts showed development only in those cultures containing both ecdysone and MF.

Table II summarizes a further series of experiments in which the agents were administered sequentially. For this purpose, individual testes were cultured for 1 hour in a medium containing ecdysone but no MF. They were then thoroughly rinsed and cultured for 7 days in medium containing MF but no ecdysone. In

TABLE II

*Spermatogenesis in intact testes of Cynthia pupae cultured with or without addition of MF and α-ecdysone**

Preincubation for 1 hour with:		Culture for 7 days with:	Number of testis cultures	Number of testes showing spermatogenesis	Developing cysts in responding testis (%)
—		MF	30	0	0
—		Ecdysone	21	0	0
—	Rinsed	Ecdysone + MF	30	30	40–80
Ecdysone		MF	12	12	20–50
MF		Ecdysone	12	0	0
Ecdysone + MF		No MF, no ecdysone	12	0	0

* α-Ecdysone, when added, was at a concentration of 1.6 μg per 100 μl medium.

parallel experiments the two agents were administered in reverse order. The results as summarized in Table II were clear-cut: development took place only when ecdysone came first.

In a final group of experiments the testes were cultured for one hour in medium containing both ecdysone and MF. They were then rinsed and cultured for 7 days in a medium lacking both ecdysone and MF. As indicated in Table II, no development took place.

3. *Ecdysone titer in relation to spermatogenesis*

Intact testes of diapausing Cynthia pupae were cultured in MF-containing plasma to which graded doses of α-ecdysone were added. After 7 days the testes were torn open and the development of the germinal cysts scored in the usual way.

As summarized in Table III, significant development invariably took place when the 100 μl of medium contained not less than 0.01 μg of α-ecdysone. The lower dose of 0.005 μg caused significant development in 2 of 4 testes, whereas the still lower dose of 0.001 μg was completely ineffective. The critical dose of 0.01 μg is equiv-

TABLE III

Spermatogenesis in intact testes of Cynthia pupae cultured seven days in plasma containing MF plus graded concentrations of α-ecdysone

Conc. of α-ecdysone (μg/100 μl)	Number of testis cultures	Number showing spermatogenesis	Developing cysts in responding testis (%)	
			Stages II and III	Stage IV
8	2	2	30–50	30–40
4	4	4	20–50	10–20
2	4	4	30–40	10–20
0.4	2	2	10–40	5–10
0.16	2	2	20–30	5–10
0.08	3	3	10–30	5–10
0.04	2	2	10–30	5–10
0.01	4	4	10–30	0–10
0.005	4	2	10–20	0–10
0.001	4	0	0	0

alent to a final concentration of one part α-ecdysone per ten million parts medium (2×10^{-7} M).

4. *Tests of other steroids and specific solvents in cultures of intact testes*

(a) *Effects of solvents.* Intact Cynthia testes were cultured in 100 μl of MF-containing plasma. Specific sterols were dissolved in one or more organic solvents, diluted to 10% by the addition of water, and 10 μl of the resulting solution administered to each culture.

In preliminary experiments of this type many organic solvents, despite their low concentration in the medium (*ca.* 1%), were found to have deleterious effects on the testes. Perhaps by injuring and thus altering the penetrability of the testis walls, the lower alcohols (see Table IV) allowed the entry of MF and the resulting onset of cyst development. These alcohols had no effect on cyst development if used in the absence of MF, whether with intact testes or with naked cysts. Solvent effects were overcome by the discovery that many of the steroids were soluble in

TABLE IV

Spermatogenesis in intact testes of Cynthia pupae cultured six days in plasma containing MF plus specific steroids or solvents†*

Additives	Number of testis cultures	Number showing spermatogenesis	Developing cysts responding testis (%)
None (control)	32	0	0
Methanol	2	Dead	
Ethanol	1	1	10–20
1-Propanol	2	2	5–10
2-Propanol	10	8	20–30§
Dioxane	10	2	10–20§
1,2-Propanediol	26	0	0
α-Ecdysone	32	32	30–80
β-Ecdysone	16	16	30–60
Cyasterone	8	8	20–40
Inokosterone	6	6	30–60
Ponasterone A	6	6	20–50
Ponasterone C	6	6	30–60
Rubrosterone	10	6	5–10§
"Triol"‡	10	0	0
Cholesterol	6	0	0
Estradiol-17β	4	0	0
Estriol	4	0	0
Progesterone	4	0	0
Aldosterone	4	0	0
Deoxycorticosterone	4	0	0

* The steroids were administered in concentrations of 0.5–2 μg per 100 μl medium.
† Solvents administered in final concentrations of *ca* 1%.
‡ 5β-cholest-7-en-one,2β,3β,14α trihydroxy.
§ Developed not beyond Stage II.

1,2-propanediol (propylene glycol) and that this solvent had no detectable influence on the cultures when present in a final concentration of 1%.

(b) *Effects of phytoecdysones.* As summarized in Table IV, β-ecdysone, cyasterone, inokosterone, ponasterone A, and ponasterone C were able to duplicate the effects of α-ecdysone on the intact testes. This result is of special interest since all these materials are known to be highly active in provoking adult development when injected into diapausing Cynthia pupae (Williams, 1968).

(c) *Effects of rubrosterone and "triol."* These two materials are known to be inactive when physiological doses were assayed by injection into diapausing Cynthia pupae (unpublished observations of C. M. W.). In the cultures of intact testes, the triol was inactive and rubrosterone showed only a trace of activity.

(d) *Effects of cholesterol and of mammalian hormones.* Since these materials (see Table IV) were insoluble in 10% 1,2-propanediol, a weighed amount of each was dissolved in absolute ethanol and an appropriate volume placed in a sterile centrifuge tube. The ethanol was evaporated in a stream of nitrogen and the material redissolved in MF-containing plasma. The latter was then used to prepare the cultures.

As indicated in Table IV all these materials were inactive when assayed on intact testes. They are also known to be inactive in ecdysone assays carried out on diapausing saturniid pupae (unpublished experiments of C. M. W.).

5. *Effects of simultaneously cultured brains and/or prothoracic glands*

The ecdysone requirement, as indicated in Table III, is fully satisfied when as little as 0.01 μg of α-ecdysone is added to the 100 μl of MF-containing medium in each culture. We sought to determine whether this critical level of ecdysone activity can be generated *in vitro* by the culture of appropriate endocrine organs. Attention focused on the brain and prothoracic glands since a long-standing, albeit unproven, principle of insect endocrinology is that ecdysone is synthesized and secreted by the prothoracic glands when the latter are activated by a hormone secreted by the brain (Williams, 1947, 1952; Possompès, 1953; Wigglesworth, 1952, 1957, 1964).

Previous studies of the *in vivo* activities of these organs in saturniid silkworms were helpful in the design of the present experiments. Thus, on the basis of this knowledge, the optimal sources of active prothoracic glands (*i.e.*, glands already activated by brain hormone) are mature larvae on the first day of cocoon construction (Williams, 1952) or, alternatively, diapausing pupae of *Antheraea polyphemus* or *A. pernyi* after storage at 5° C for longer than 10 months. In the case of potentially polyvoltine species such as *Samia cynthia, A. polyphemus*, and *A. pernyi*, brains and prothoracic glands in the inactive condition can be obtained from freshly pupated individuals reared under the short-day conditions which provoke the onset of pupal diapause (Williams and Adkisson, 1964). By contrast, the brains of these same species are active in freshly pupated individuals reared under the long-day conditions which avert the onset of diapause (Williams, 1969). In all species, a further routine source of active brains are diapausing pupae stored at 5–8° C for 3 months or longer (Williams, 1956). In the experiments reported here we have assumed that the activities of the brains and prothoracic glands *in vitro* were the same as the above-mentioned activities *in vivo*.

Table V

Cultures of intact Cynthia testes in MF-containing plasma: effects of simultaneously cultured Cynthia brains and/or prothoracic glands

Endocrine organs	Donors	Number of cultures	Number that developed	% Developing cysts in responding testes
[None]	[Controls]	15	0	0
Active brain	Non-diapausing pupae	9	0	0
Inactive prothoracic glands	Diapausing pupae	9	0	0
Active prothoracic glands	Larvae (1st day of spinning)	10	9	30–80
Inactive prothoracic glands plus inactive brain	Diapausing pupae	6	0	0
Inactive prothoracic glands plus active brain	Brains from non-diapausing pupae; prothoracic glands from diapausing pupae	6	4	10–40
Active prothoracic glands (homogenized)	Larvae (1st day of spinning)	6	0	0

Individual testes of diapausing Cynthia pupae were cultured in 100 μl of MF-rich plasma derived from Cynthia pupae that had been stored at 25° C for 5 to 6 months. After 3 to 6 days of incubation at 25° C, the testes were torn open and examined microscopically.

As summarized in Table V, no development took place in 15 control cultures. The same negative results were observed in cultures supplemented with either an active brain or a pair of inactive prothoracic glands. The combination of inactive brain plus inactive prothoracic glands was also ineffective. By contrast, nearly all

Table VI

Cultures of intact Cynthia testes in MF-containing plasma: effects if simultaneously cultured organs from two other saturated species

Endocrine organs	Donors	Number of cultures	Number that developed	% Developing cysts in responding testes
[None]	[Controls]	33	0	0
Thoracic or abdominal ganglia	Pernyi pupae (prolonged chilled)	12	0	0
Active brains	Pernyi or Polyphemus pupae (chilled 5 months)	25	0	0
Inactive prothoracic glands	Pernyi pupae (chilled 5 months)	3	0	0
Active prothoracic glands	Pernyi or Polyphemus pupae (prolongly chilled)	17	14	30–50
Inactive prothoracic glands plus active brain	Pernyi pupae (chilled 5 months)	2	2	50–80
Active prothoracic glands plus active brain	Polyphemus pupae (prolongly chilled)	7	7	50–80
Active prothoracic glands (sonicated)	Polyphemus pupae (prolonged chilled)	9	0	0

testes showed clear-cut spermatogenesis when cultured in the presence of active prothoracic glands or inactive prothoracic glands plus active brains.

Table VI summarizes additional experiments in which the testes of *Samia cynthia* were cultured in MF-rich Cynthia plasma along with organs obtained from pupae of two other saturniid species. Here again, spermatogenesis took place only when the cultures contained active prothoracic glands or inactive prothoracic glands plus active brains.

As recorded in the bottom line of Table VI, pairs of active prothoracic glands were ineffective when killed by sonication. In Table V a similar negative result is noted for active prothoracic glands that had been homogenized.

Discussion

Spermatogenesis in cultures of intact testes requires the presence, not only of MF, but also of ecdysone (Table II). By contrast, the germinal cysts, when removed from the testes and cultured in direct contact with MF-containing medium, do not require ecdysone (Kambysellis and Williams, 1971). These findings can fully account for the developmental reactions within the testes of normal or injured pupae (Table I). Thus, notwithstanding the presence of high titers of MF in the hemolymph, diapausing pupae show no trace of spermatogenesis until such time as ecdysone is injected or secreted by the prothoracic glands.

Particularly illuminating are the experiments summarized in Table II in which intact testes were subjected to the simultaneous or sequential administration of ecdysone and MF. Spermatogenesis took place only when the exposure to ecdysone either accompanied or preceded the exposure to MF. It is also of interest that the effects of ecdysone were persistent after 1 hour of treatment, whereas exposure to MF for at least 24 hours was prerequisite for the initiation of the developmental response (Kambysellis and Williams, 1971).

Evidently, the function of ecdysone is to alter the penetrability of the testis walls and thereby to facilitate the entry of MF and perhaps other blood-borne molecules into contact with the germinal cysts. In support of this "permissive" role of ecdysone we found that when testes were cultured in an ecdysone-free but MF-containing medium, the development of the germinal cysts could be provoked by puncturing the testes or by the addition of certain organic solvents which apparently damage the testis walls (Table IV).

Otherwise, the effects of ecdysone on intact testes was specific for α-ecdysone, β-ecdysone, and phytoecdysones known to be highly active *in vivo* (Williams, 1968). α-Ecdysone, as illustrated in Table III, was fully effective when administered in concentrations as low as 2×10^{-7} M.

Of further interest in this connection is a series of experiments (to be described in detail elsewhere) in which intact testes were cultured for 2 hours in the presence of very low doses (2×10^{-9} M) of tritiated α-ecdysone. The label was rapidly taken up by the testes and only a small fraction (15%) could be eluted into the medium when the testes were cultured for 12 hours in the absence of ecdysone. At the conclusion of the experiment 75% of the radioactivity was recovered in extracts of the testis walls, even though the walls account for a much lower fraction of the mass of the testis.

This selective binding, presumably to ecdysone "receptors" (Cherbas and

Cherbas, 1970), can obviously account for the long-lasting effects of even brief exposure to ecdysone (Table II). However, at the present time we are unable to state where this binding takes place in relation to the numerous cellular and membranous components of the testis walls. There is also insufficient information to decide whether the changes in penetrability involve the active or facilitated transport of MF, or the opening of channels for passive diffusion between cells.

In the absence of added ecdysone, the required level of ecdysone activity can be generated *in vitro* by the simultaneous culture of living, endocrinologically competent prothoracic glands (Tables V and VI). Since this activity failed to appear in the absence of activated prothoracic glands, these findings strongly support the view that the prothoracic glands synthesize and secrete one or more materials with ecdysone activity. The only reasonable alternative is that the prothoracic glands secrete an ecdysone precursor which can be converted into active hormone by the plasma or the reacting tissues—in this case the testis itself.

We have observed that active prothoracic glands were ineffective when sonicated or homogenized. This implies that the synthesis and secretion of ecdysone are synchronized and that little hormone is stored within the prothoracic glands. On the basis of the calibration of the *in vitro* system (Table III) the "stored" ecdysone in a pair of active prothoracic glands can be equated to less than 0.005 μg α-ecdysone.

As summarized in Tables V and VI, prothoracic glands which were known to be inactive *in vivo* were also inactive *in vitro*. However a most noteworthy finding was that inactive glands could be "turned on" by the addition to the culture of a living, endocrinologically competent brain.

Our interpretation of the experiments reported here as well as in the previous paper is summarized in Figure 1. The germinal cysts within the intact testis re-

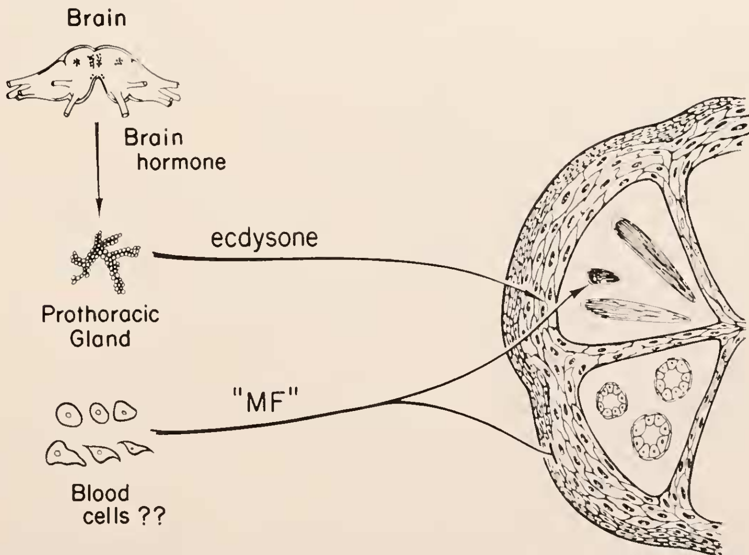

FIGURE 1. A diagrammatic representation of the control of spermatogenesis. See text for detailed description.

quire MF for their metamorphosis into bundles of spermatozoa. Though MF is ordinarily present in the surrounding hemolymph, it can gain access to the cavities of the testes only when the penetrability of their walls is altered in response to ecdysone.

MF is apparently synthesized and secreted into the blood plasma by one or more types of hemocytes when the latter are activated, for example, in response to injury of the pupal integument. Thus, as reported previously (Kambysellis and Williams, 1971), MF activity can be generated *in vitro* by culturing activated hemocytes in MF-free medium.

Finally, as diagrammed in Figure 1, the results of the present study show that ecdysone activity can be generated in an *in vitro* system containing living, endocrinologically active prothoracic glands. Moreover, endocrinologically inactive glands can be "turned on" *in vitro* by the further addition of living brains which are competent to secrete brain hormone.

The molecular mechanism of ecdysone action on the target cells of the testis walls remains, for the time being, a mystery. By analogy to what is known about the mode of action of the sterol hormones of vertebrates (Edelman and Fimognari, 1968; Gorski *et al.,* 1968; Fang *et al.,* 1969; Jensen *et al.,* 1969, Jensen *et al.,* 1971; O'Malley *et al.,* 1970; Steggles *et al.,* 1971), we would not be surprised to learn that the change in penetrability involves the *de novo* synthesis of one or more proteins.

Effects on permeability, including active transport and kindred phenomena, have often been cited as a mechanism for the implementation of hormone action (for review see Turner and Bagnara, 1971). However, in virtually all cases the altered permeabilities have pertained to water, ions, sugars, or other small molecules. Kroeger (1968) has presented evidence that ecdysone affects permeability relationships in the salivary glands of larval *Chironomus.* The effects in this case are thought to be on the active transport of sodium and potassium ions between nucleus and cytoplasm. The testicular system studied in the present investigation is therefore remarkable in that the ecdysone-induced change in penetrability is for a macromolecule which, in itself, possesses biological activity.

It is of interest that mammalian spermatozoa are known to differentiate in a fluid whose composition differs from that of the blood or lymph. The seminiferous tubules are surrounded by a "blood-testis barrier" which is virtually impermeable to serum albumin, inulin, and even to such small molecules as galactose and glutamic acid (Setchell *et al.,* 1969; Setchell, 1970; Dym and Fawcett, 1970).

It is tempting to speculate that penetrability barriers analogous to those of the testes may exist elsewhere in the insect body associated with cellular and extracellular layers such as the sheath of the nervous system or the basement membranes and mucopolysaccharide coatings of many cell types (for reviews see Ashhurst, 1968; Smith, 1968). Whether the penetrabilities of these other blood-tissue barriers may also be affected by ecdysone can be decided only on the basis of further study.

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Summary

1. Germinal cysts, when removed from the testes and cultured in direct contact with the medium, undergo meiosis and spermatogenesis provided that a "macromolecular factor" (MF) is present.

2. By contrast, spermatogenesis within intact testes derived from either normal or injured pupae requires the presence, not only of MF, but also of ecdysone. The same is true for spermatogenesis *in vivo*.

3. The critical effects on the testes were exerted by concentrations of α-ecdysone as low as 2×10^{-7} M. The effects were shown to be specific for α-ecdysone, β-ecdysone, and phytoecdysones known to be highly active *in vivo*.

4. When MF and ecdysone were administered sequentially to cultures of intact testes, spermatogenesis took place only when the exposure to ecdysone preceded the exposure to MF.

5. The ecdysone requirement can be satisfied by the simultaneous culture of a pair of living, activated prothoracic glands. The glands were ineffective when homogenized or killed by sonication.

6. Prothoracic glands known to be inactive *in vivo* were also inactive *in vitro*. However, they could be "turned on" *in vitro* by the addition to the cultures of pupal brains known to be competent to secrete brain hormone.

7. These results strongly support the view that the prothoracic glands are activated by brain hormone to synthesize and secrete one or more materials with ecdysone activity.

8. Present indications are that ecdysone plays a permissive role in spermatogenesis and that its sole function is to alter the penetrability of the testis walls and thereby facilitate the entry of MF and perhaps other blood-borne molecules into contact with the germinal cysts.

9. Consideration is given to the possibility that the permissive role of ecdysone may not be an exclusive property of the testicular system.

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